Blood Group and Crossmatch: Issues and Troubleshoots

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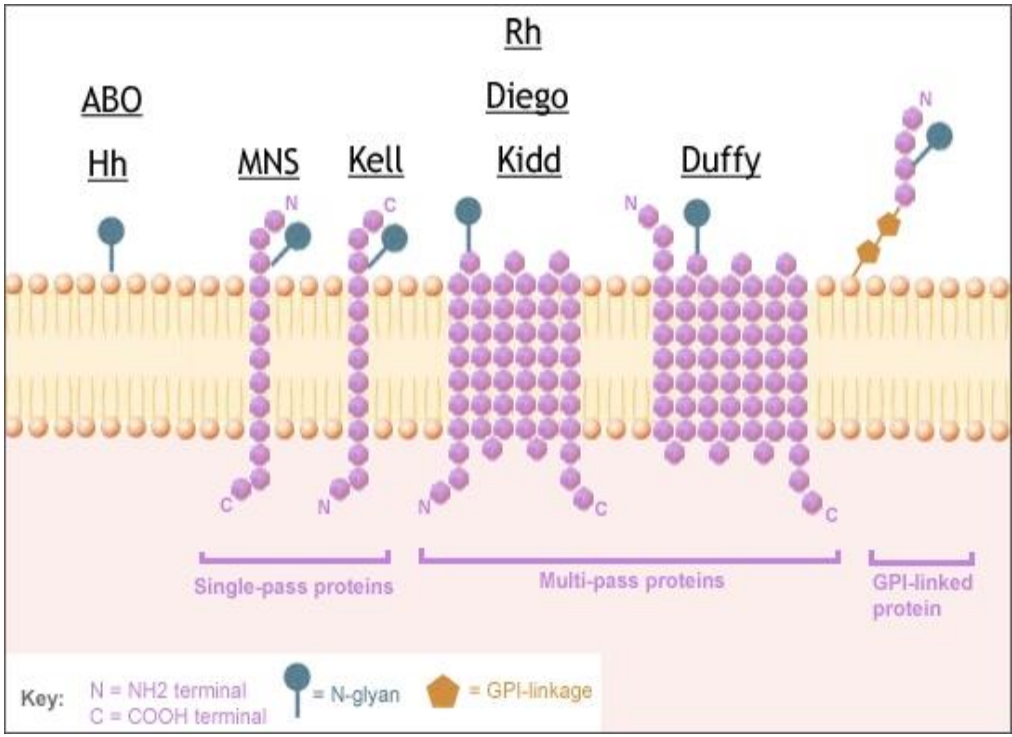
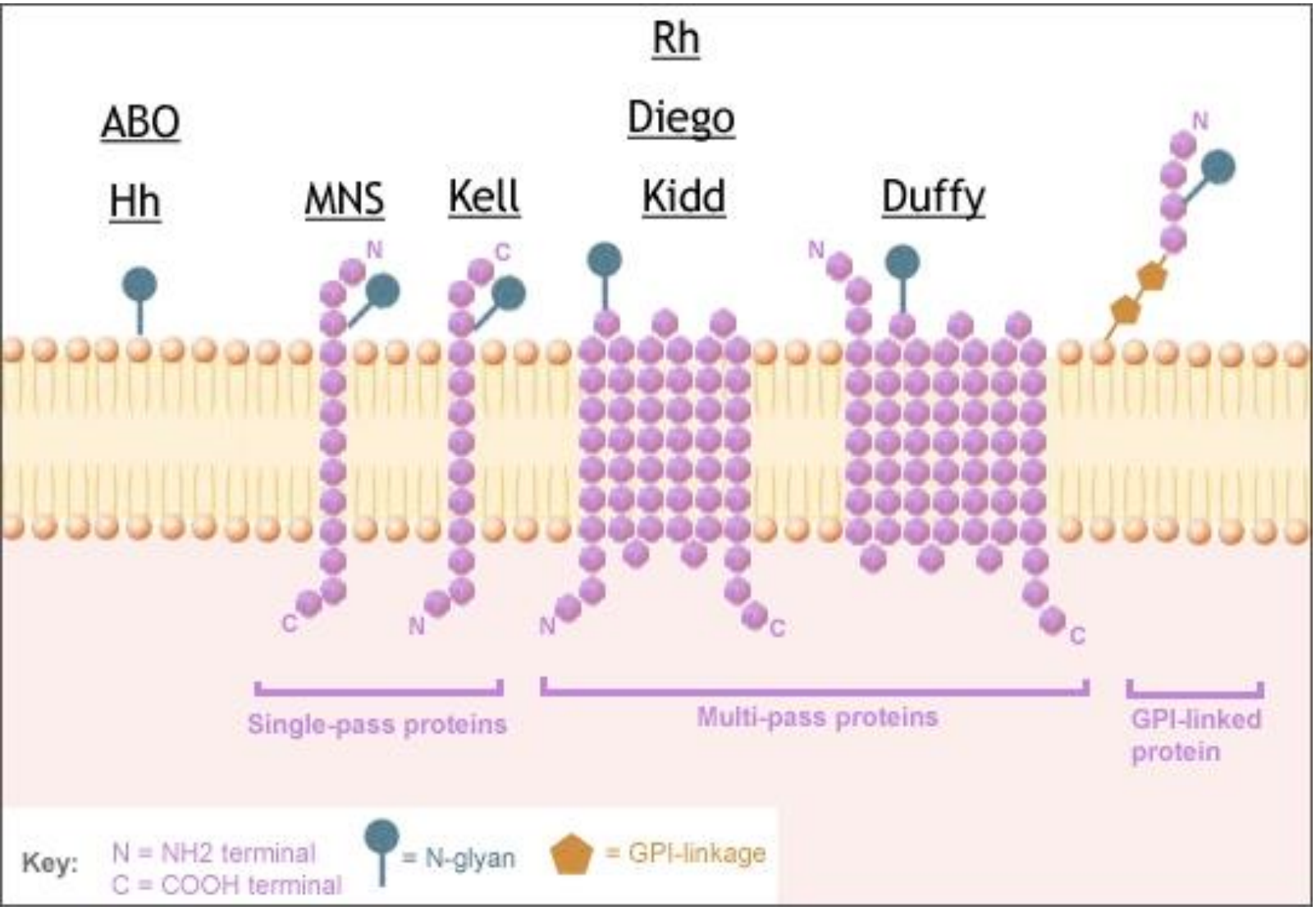
Introduction:
Blood Group Systems

- Karl Landsteiner discovered ABO system - in 1900
- ABO system remains the most significant system till date
- Rh is 2nd most important system after ABO
  - Discovered in 1940

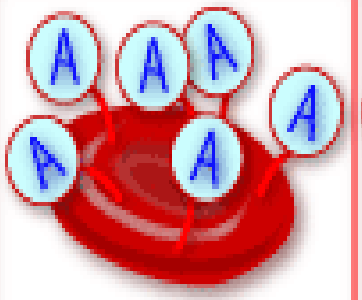
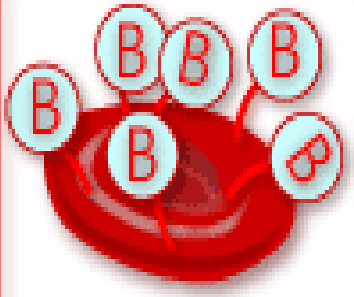
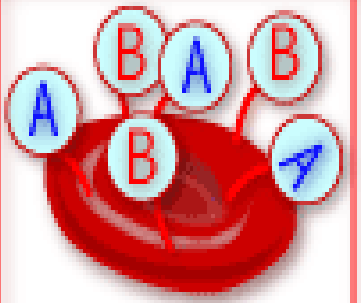
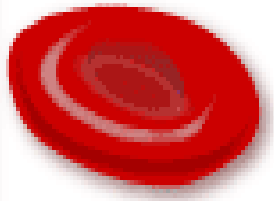
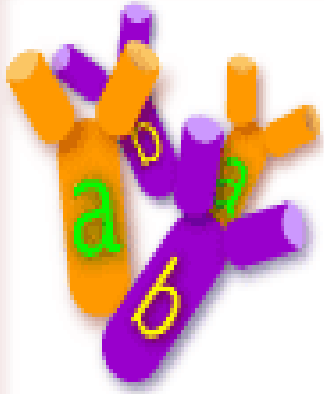
Introduction : Blood Group System

- Blood group antigens are on RBC
- 35 blood group system known
- ABO & Rh most important
- Others are
  - Kell,
  - Duffy,
  - Kidd,
  - P,
  - MNS etc




Rh
ABO
Diego
Hh
MNS
Kell
Kidd
Duffy
N
C
Single-pass proteins
Multi-pass proteins
GPI-linked protein
Key:
N = NH2 terminal
C = COOH terminal
= N-glyan
= GPI-linkage

The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	 No agglutinin	 a and b agglutinin

Laboratory Determination of the ABO system

Laboratory testing for ABO

- Detection of Antigen on Red cell surface

Cell grouping

- Red cells with unknown antigen tested with known antisera
- Using commercial reagents
  - Anti-A
  - Anti-B

- Detection of Antibodies in plasma

Serum grouping

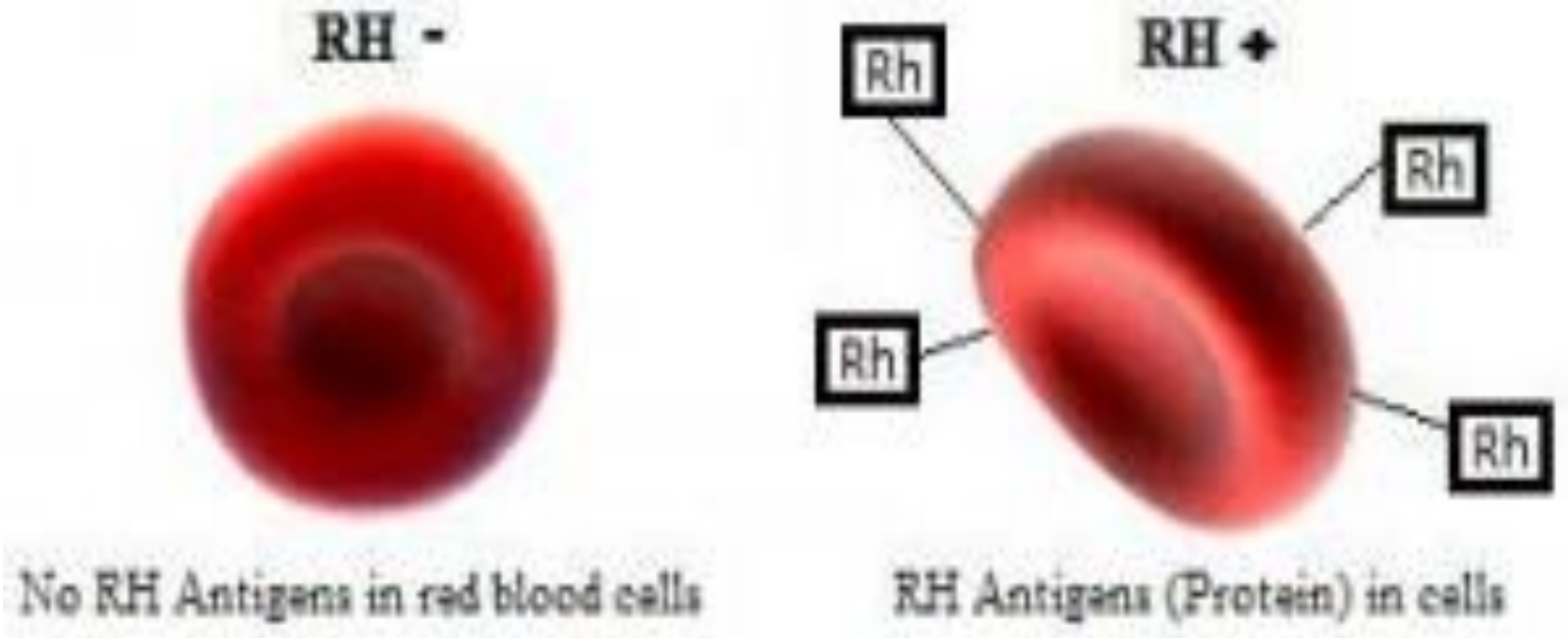
- Serum with unknown antibodies tested with known antigens
- Using reagent red cells
  - A cells
  - B cells

Reaction pattern of ABO group (Cell grouping & Serum grouping)

Red cells tested with			Serum tested with			Interpretation
Anti -A	Anti -B		A cells	B cells	O cells	
4 +	0		0	4 +	0	A
0	4 +		4 +	0	0	B
4 +	4 +		0	0	0	AB
0	0		4 +	4 +	0	O

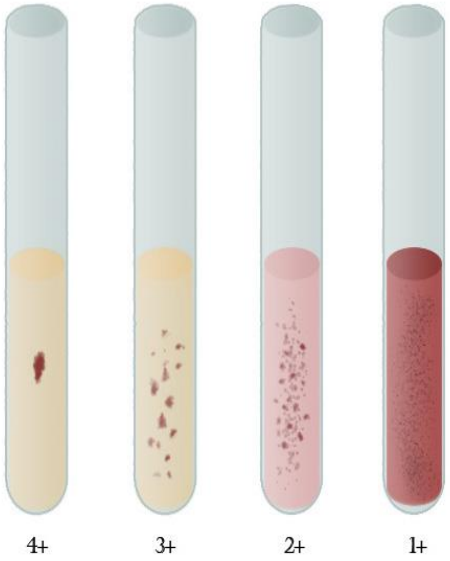
Laboratory testing for Rh

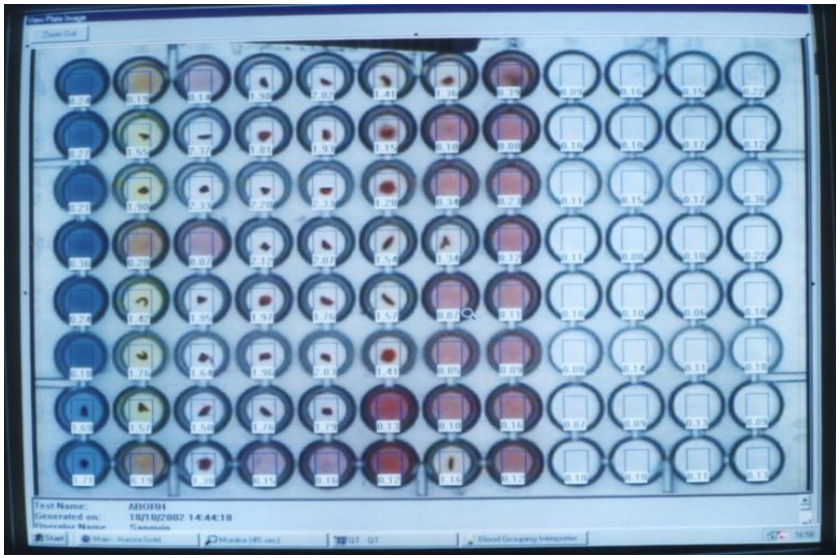
- D antigen is most immunogenic
- Routine testing for D antigen
- Using commercial Antisera (Anti-D)
  - Rh Positive
  - Rh Negative

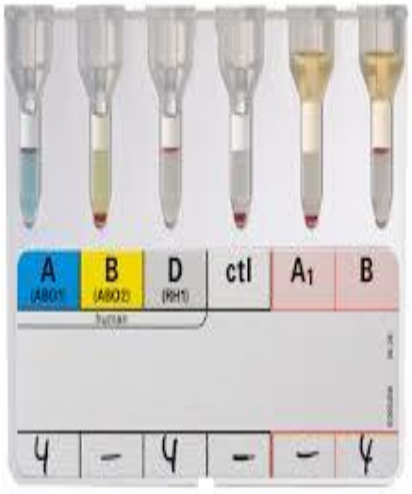


Techniques

- Tube technique
- Microplate technique
- Column agglutination technique







Anti-globulin test (AHG)

The anti-globulin test also called Coomb's test in honor of one of the investigator who developed the test for laboratory use in 1945 for detecting attachment of antibodies that didn't produce agglutination.

This test uses antibodies to human globulins.

It was first used to demonstrate antibodies in serum but later the same principle was used to demonstrate in –vivo coating of red cells with antibody or complement components.

Antihuman globulin test

Coombs' test

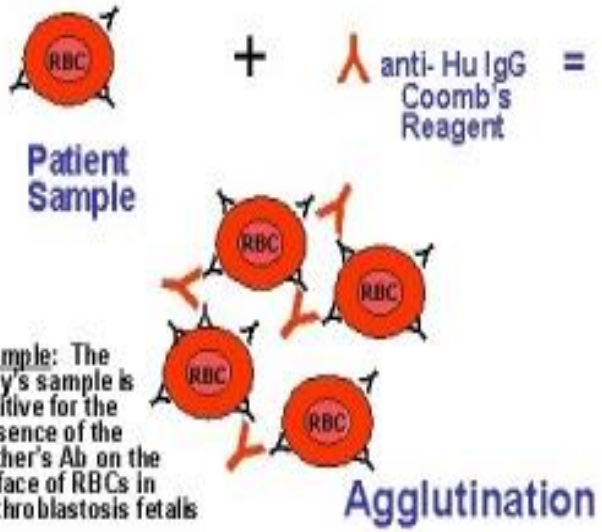
Principle:

The AHG acts as bridge and induces agglutination of sensitized red cells

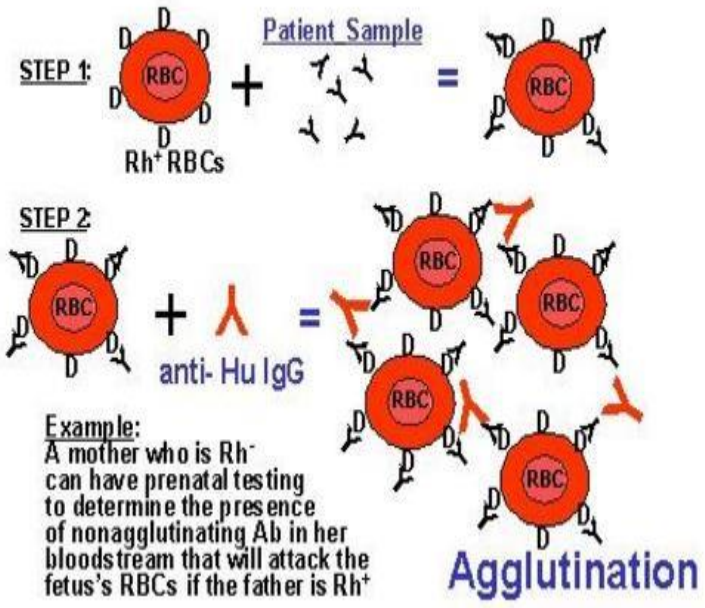
DAT : to demonstrate in vivo sensitization

IAT: to demonstrate in vitro sensitization

DIRECT COOMB'S TEST



INDIRECT COOMB'S TEST



Issues and Troubleshoots in Routine Blood Grouping

Importance

- ✓ It is important to perform blood group correctly
- ✓ Wrong grouping results could be life threatening to patients

Identify the problem

- Most of the time, the problem is technical
  - Mislabeled tube
  - Failure to add reagent
  - Either repeat test on *same* sample, request a new sample, or wash cells
- Other times, there is a *real discrepancy* due to problems with the patient's red cells or serum

Issues in Blood Grouping

ABO grouping problems

- Discrepancy in Cell and Serum Grouping

Rh grouping problems

- Weak D/Partial D

ABO Grouping problems

When cell and serum grouping do not match

Which one is right ??

Interpretation ??

Such situations are called discrepancy

Important to note :

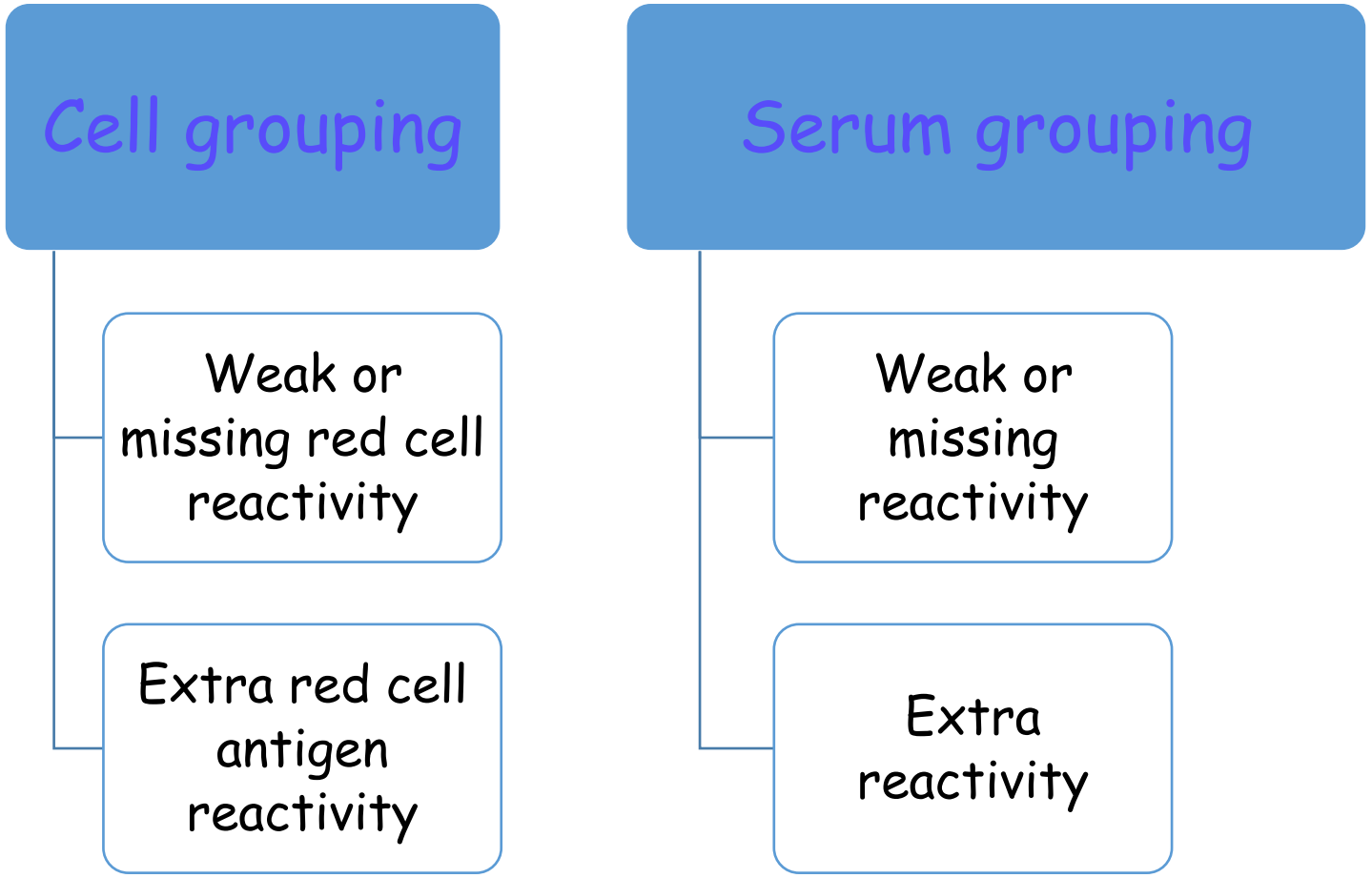
Discrepant results must be recorded

Interpretation must be delayed till discrepancy resolved

If donor sample : do not release the unit

If patient sample : If transfusion is urgent group O unit may be issued

Discrepancy in ABO grouping



To resolve

- Repeat the test with proper technical procedure
- Ask for fresh sample, repeat the test
- Check medical history, diagnosis, age, previous transfusion
- Perform additional tests
- Repeat after washing red cells, change cell:serum ratio, increase incubation time
- Adsorption Elution
- Secretory status

Interesting problem cases:

- 21 yr /F , Clinical diagnosis AML, M1, Blood group results

Anti-A	Anti-B	Ac	Bc	Interpretation
0 to 1+	0	0	4+	? A ? Subgroup of A

- Cell grp- weak reaction for A ag
- Serum grouping- A group
- Possibilities :
  - Subgroup of A
  - Weakening of A ag due to disease

- To resolve:
  - ✓ Previous bld grp report if kn
  - ✓ Detail clinical history
  - ✓ Special techniques

Interesting problem cases:

- 2 months /M, posted for Surgery on next day
- Blood group results

Anti-A	Anti-B	Ac	Bc	Oc	Interpretation
0	4+	0	0	0	? B ?AB

- Cell grp- B
- Serum grouping- AB
- Possibilities:
  - Weak antibodies
  - ✓ Newborn:
  - ✓ Old age:
  - ✓ Hypogammaglobulemia

To resolve:

- ✓ Check age of the pt
- ✓ Clinical diagnosis
- ✓ Modification of techniques -extended incubation, alter cell serum ratio etc

Interesting problem cases:

- F/43, T cell lymphoma, Hb 5.6
- Blood group results

Anti-A	Anti-B	Ac	Bc	Oc	Interpretation
0	3+	3+	3+	3 +	? Irregular Antibodies

- Alloantibodies
- Autoantibodies
- Others : abnormal proteins, fibrin clot, recent infusion of immunoglobulins etc

To resolve:

- ✓ Alloantibodies: identification by using reagent red cell panel
- ✓ Auto antibodies: test at different temperature, prewarm technique,
- ✓ Abnormal high proteins: alter cell to serum ratio

Interesting problem cases:

- 10 yr M, case of NHL, Hb 7.0, on chemotherapy

Anti-A	Anti-B	Ac	Bc	Oc	Interpretation
0	0	3+	3+	3 +	? O ? Bombay

To confirm:

1. Test with Anti-H
2. Test with various batches of anti-A, anti-B, anti-AB, anti-H
3. Family study
4. Secretor status

Bombay Phenotype (Oh)

- ✓ Discovered in Bombay by Bhende et al in 1952
- ✓ Absence of A, B and H antigen
- ✓ Presence of anti-A, anti-B & anti-H
- ✓ Should be transfused only with Bombay blood group

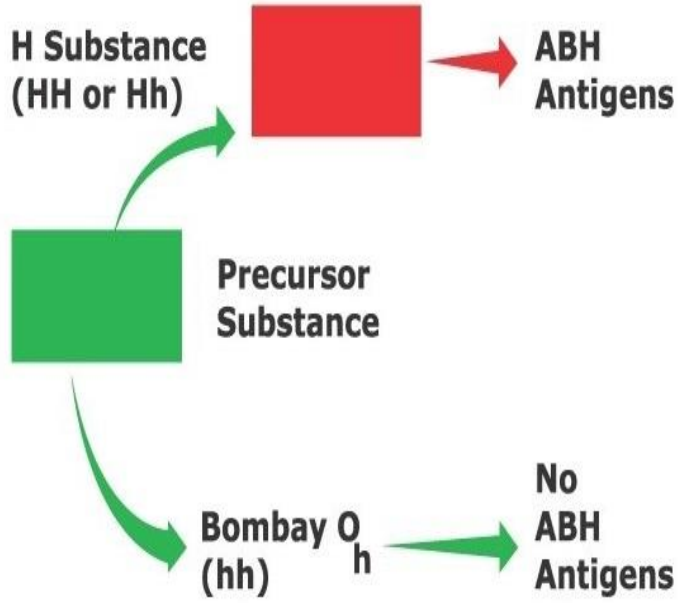
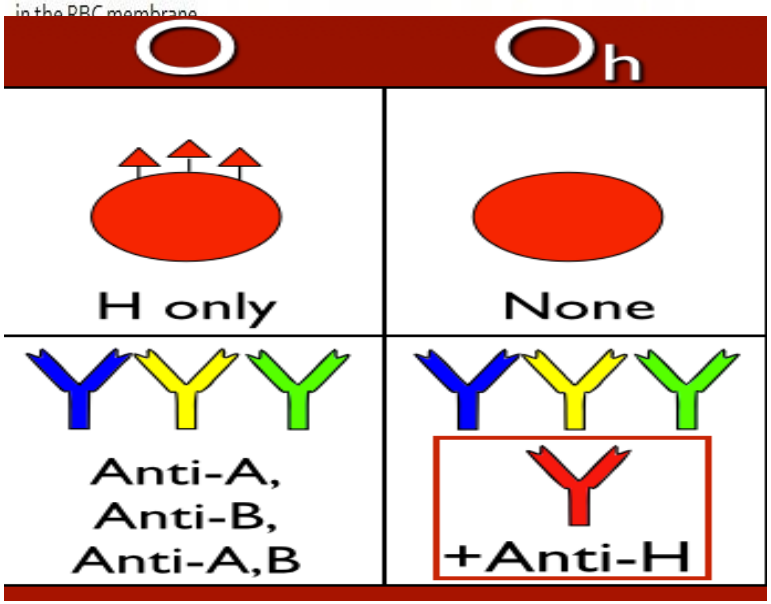


Fig:The biosynthesis of the H antigen and the A and B antigens involves a series of enzymes (glycosyltransferases) that transfer monosaccharides. The resulting antigens are oligosaccharide chains, which are attached to lipids and proteins that are anchored in the RBC membrane



Rh typing problems

- All Rh negative samples are tested for weak D
  - Weak D :
    - extended incubation and test with AHG
- Significance in donor and patient

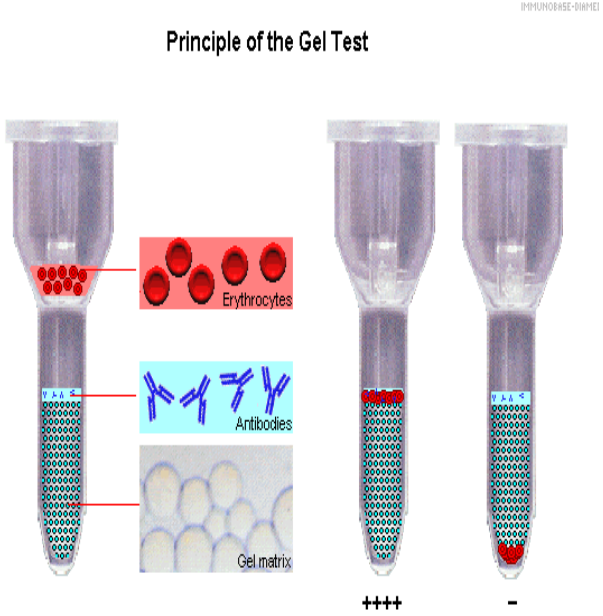
Compatibility Testing

Compatibility testing

- Set of procedures required before blood can be issued
- To make sure that there are no antibodies present in patient serum which react with donor red cells
- This is the final check on compatibility between donor & recipient
- It includes:
  - ABO & Rh grouping of Patient & Donor
  - Screening for irregular antibodies
  - Cross-matching

Techniques for compatibility

- Routine procedure
  - Saline RT & 37^0 C
  - Antiglobulin test 37^0 C
- Method
  - Test tube
  - Column agglutination



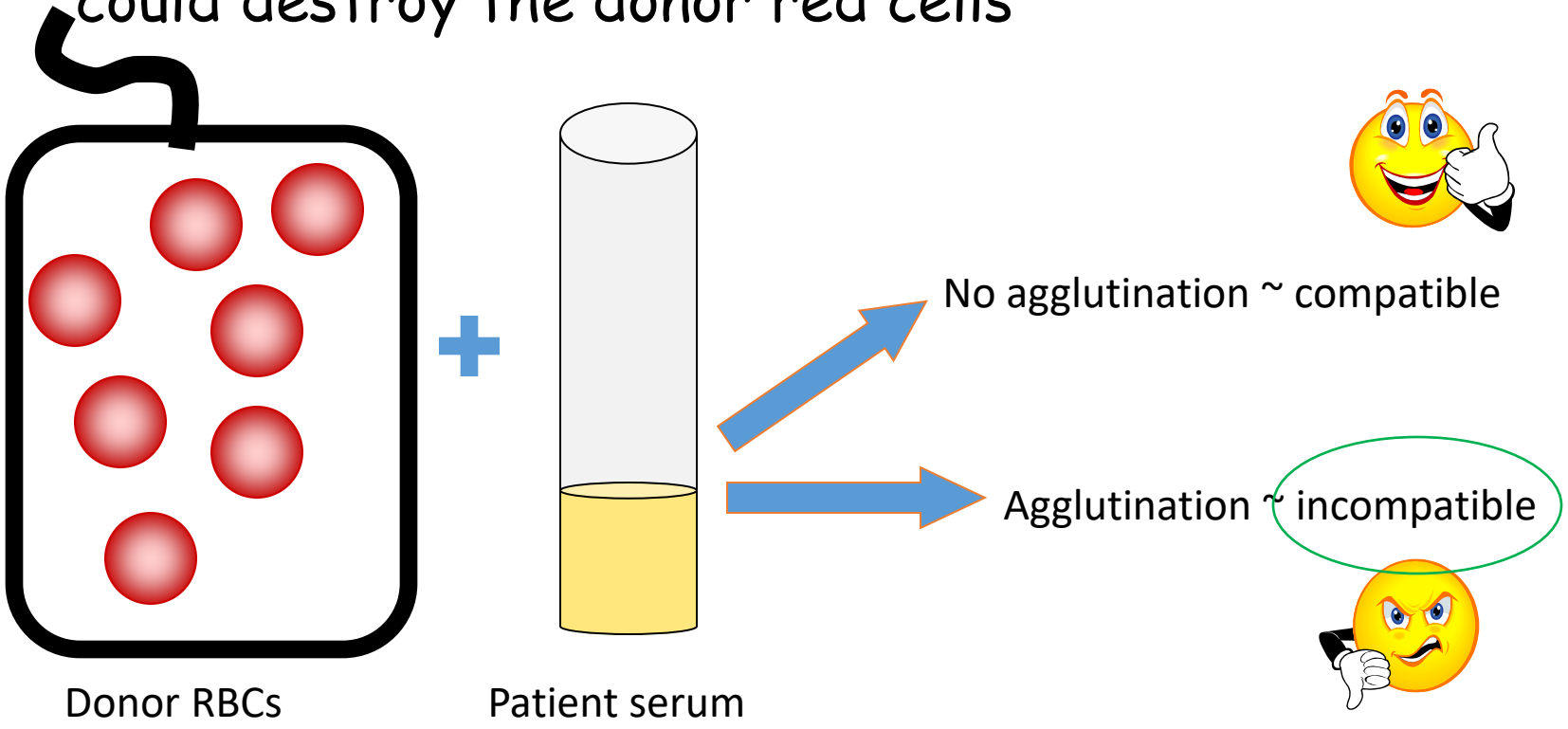
Testing of patient sample

- Verification of previous result
- If discrepancy – obtain new sample
- ABO grouping – most critical step
- Rh typing – most critical step

Issues related to Compatibility testing

Crossmatch

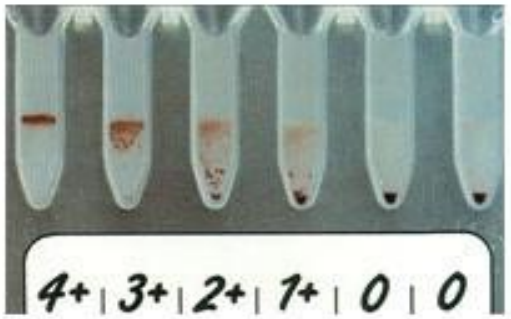
- Primary objective of crossmatch is to detect presence of antibodies in recipient's serum, which could destroy the donor red cells



Resolving incompatibilities

Causes of positive crossmatch results are

- ✓ Incorrect ABO grouping of patient or donor
- ✓ Presence of alloantibodies in patient's serum
- ✓ Presence of autoantibodies
- ✓ Abnormalities in patient serum
- ✓ Prior coating of donor red cells
- ✓ Contaminants in the test system



Incorrect grouping of patient or donor

- Due to procedural error
- Sampling error
- Repeat the blood grouping on patient and donor

  sample
- If require ask for new sample and also check

  blood group in previous record

Presence of Alloantibodies

- Antibody screening positive
- Incompatible with many donor unit
- Detail clinical history
- DAT, IAT and autocontrol
- Antibody identification
- Find out antigen negative unit

Presence of autoantibody

- ✓ Autocontrol positive
- ✓ Test at different temperature
  - ✓ (RT, 37^0c, 4^0c)
- ✓ DAT, IAT
- ✓ Titre of antibody
- ✓ Auto adsorption- to remove the autoantibodies- perform compatibility

Abnormalities in patient's serum

- Altered A/G ratio in certain disease condition-may cause RBCs to stick together giving appearance of stacks of coins- Rouleaux formation
- Mimic agglutination
- Resolved by saline replacement procedure
- High molecular weight dextrans, plasma expanders may give false positive results

Key points

- ✓ Follow standard procedures & manufacturer's instruction
- ✓ Use appropriate equipment and reagents
- ✓ If there is discrepancy
- ✓ Repeat test on same sample
- ✓ Still it persists
- ✓ Obtain clinical diagnosis, previous bld grp report, transfusion history, medication
- ✓ Obtain fresh sample
- ✓ Review results of allo or auto antibodies

Thank You !!!